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STUDIES IN THE MECHANISM OF ANTIBODY FORMATION WITH SPECIAL REFERENCE TO ANTIBODY PRODUCTION PROMOTING FACTOR (A.P.P.)

PART I. INFLUENCE OF THE A.P.P. ON THE PRECIPITIN PRODUCTION IN RABBITS

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CHAPTER 1. INTRODUCTION

In recent years, much progress has been made in immunology. Some scientists have made studies on cells and organs which are related to the production of antibodies, and some have tried to clarify the mechanism of their formation. Much advancement has been made in this field, and further experiments are being made on the promotion of antibody production with many different approaches. For example, an attempt to promote production of a special kind of antibody by combining an ordinary substance with a special kind of antigen is one of the studies in this field. It is impossible to mention all of the component substances of antigen which have been investigated in the past.

Pinoy[1] proved the strengthening power of vaseline and lanolin in the formation of agglutin in 1916. Later, Freund, et al.[2], reported similar results by using many different drugs and microorganisms. Studies also have been made on the effect of extracts of living organs and their suspensions on the formation of antibodies. Ohashi and Watanabe[4] reported that alcohol extract of lymphatic gland of a splenectomized rabbit promoted the formation of antibody. Matsuo[5,6] reported that salt water extract of bovine lymph gland had no influence on the formation of agglutinin in healthy rabbits, but it restored the ability of antibody production in a rabbit which had lost it due to improper functioning of the

reticulo-endothelium system. Miyake[7] stated the existence of a certain process which helps the production of immune substance in lymph tissue lactis which contains antigen. Ninomiya and Chikushi[8] reported that administering lymph gland extract through the mouth of a rabbit which had splenectomy showed no effect on the production of hymolysin.

Hiraki and Naito[9] recognized the existence of a substance in salt water extract of bone marrow which, when administered to rabbits, promotes the formation of both serum precipitin and agglutinin. Ohashi and Watanabe reported that alcohol extract of bone marrow increased agglutinin production in the early stage after having been administered to rabbits, but was negligible in later stages.

Many workers recognized the existence of a certain kind of antibody formation promoting factors in liver[10,11,12,13,14,15,16,17], in spleen[4,8,13,14,15,16,18,20,21,22,23,24,25], in kidney corpuscle[10,15,16], in thyroid gland[32], in petuitary tissue[26], in testicle[14,15,16], in thymus gland[26,32], in testis corpuscle[14,15,16], in kidney[10,14,16], in lung[34], in tonsil[27], in alimentary tube, in duodenum membrane[33], in muscle, in white corpuscle[29,30], and in blood[28]. Most of their work was limited to living body organs. Most of these studies concentrated on finding the influence of these substances as complements. Their main emphasis was on the antibody production promoting factors and prolongation of their function. Little work, however, has been done on accelerating production of antibody and shortening its latency at the same time.

Fukase[34] suggested in 1950 that at the time when an antigen is administered into a living body and antibody formation starts for self-protection, a certain catalytic reaction occurs. He thought that in this process the normal protein producing function is changed over to the production of antibody. With this theory, he made an extensive study on various organs trying to find this substance. He found a strong antibody production promoting factor in a suspension of lymph gland extracted by the supersonic wave method, and named it the A.P.P.

According to Fukase, when the A.P.P. was introduced into rabbits with typhus vaccine, it promoted the formation of serum agglutinin, its titer reaching to maximum within 2 to 3 days. He also found that the A.P.P. had the same effect on the formation of agglutinin and hemolysin in the red

corpuscles of mountain goats. He found a similar result in the formation of an antitoxin in rabbit serum against diphtheria toxoid, and he also noticed that the latency time was greatly shortened by the A.P.P.

The objective of this experiment is to further clarify the functions of the A.P.P. by studying the influence of this substance on the production of precipitin.

For measurement of precipitin, the antigen dilution method by precipitation reaction was employed. Collier and Knoller[35] first recognized the importance of measuring precipitin by the antiserum dilution method. They reported when Solastr Papposus was immunized (7 times) with the extract of Asterias rubus, the precipitin tier stayed constant after the fifth immunization, but precipitin in the serum increased along with the number of injections. Ogata[36] and Sato[37] confirmed this fact. Sato[37] named the former precipitin titer or value, and the latter, the precipitin quantity. The relationship between these two factors has been studied and reported by Masuda[38], Mida[39,40], Matsubayashi[41], and Ogata[42]. All of these workers reported that the activities of these two factors do not necessarily parallel each other.

The present author has made a study on the influence of A.P.P. on precipitin value or titer and quantity, the results of which are reported in the following.

CHAPTER II. EXPERIMENTAL MATERIALS AND METHODS

A. Experimental Materials

1) Experimental animal. Rabbits were used for this experiment. The average weight of the animals was about 2.5 Kg, and only healthy animals were selected. They were kept in the animal room of the medical department for a few days before the actual experiment. Careful observation of the environmental conditions, feed, and whether or not they had any sicknesses such as diarrhea was made.

2) Physiological salt solution. An 0.85% salt solution using chemically pure salt in distilled water was made and sterilized.

3) Antigen. Egg white was used as an antigen. Well beaten egg white was filtered through 2 layers of aspetic gauze. With this filtrate, a 20% solution using the physiological salt water was made, and heated for 30 minutes at 60°C, stiring constantly. After filtering this solution (again using two layers of aseptic gauze), 0.5% carbolic acid was added. This preparation was kept in a dark glass bottle and stored in a refrigerated room for future use.

4) Method of preparation of the A.P.P. According to the method employed by Fukase[34], a rabbit of normal health was tested with egg white, making sure it showed negative presence of precipitin. The rabbit was then killed in an airtight chamber, and its abdominal diaphragm and lymphatic gland of the knee were immediately removed. To these, about 10 times of aseptic physiological salt water was added. This mixture was then disintegrated into small pieces in a grinding machine, and was divided into test tubes in small quantities. These tubes were treated with supersonic wave for 15 to 20 minutes under 8mm crystal plate, 100 V, power 180mA. and secondary voltage of 1,500V. It was thought that in this suspension of lymph gland, the A.P.P. may be found.

5) Methods of immunization and the collection of immunization serum. The immunization of rabbits for this experimental work was done by the use of a mixture of the A.P.P. and egg white antigen mentioned above. Two parts of the antigen and one part of the A.P.P. were mixed thoroughly. Three cc of this mixture was injected to the vein on the external ears of the rabbits three times at intervals of 3 to 6 days. To the rabbits of control, either 2 cc of egg white antigen or 1 cc of the A.P.P. was used in each injection.

6) Immunization serum. From both experimental and control rabbits, blood was collected either through their external ear vein or through the heart. Serum was separated from this blood immediately after collection. The collection of blood was started after the first immunization, continuing 16 to 59 days and amounting to 10 to 14 collections. In all cases, separation of serum was done immediately after collection. Attempts were made to use the prepared materials for experimental work as soon as possible.

B. Method of Experiment

For this experiment, a layer formation method similar to that used by Ogata[43] has been employed. For the same

immunization serums, measurements for both the value and the quantity of precipitin were made at the same time.

1) Measurement on the precipitin value -- As needed, egg white solution which was used as antigen was diluted with 25 times of physiological salt water, and this solution was again diluted to double volume. This solution was introduced into small test tubes containing serum which had been diluted to three times. After having made occasional observations on these test tubes, a final observation was made after letting them stand for 5 hours at room temperature. The formation of white turbid precipitation at the juncture of the two solutions with the antigen solution of the highest dilution was used as the precipitin value (titer).

2) Method of measurement of precipitin quantity -- The above immunization serum solutions were diluted to double the amount, and they were introduced into precipitation tubes which contained 20% egg white. These tubes were kept at room temperature for observation. The formation of white precipitation at the juncture of the two solutions with the serum solution of the highest dilution after standing for 5 hours was considered as the quantity of precipitin.

CHAPTER III. THE EXPERIMENTAL RESULTS

A. The Influence of the A.P.P. on the Value of Egg White Precipitin

1. Results of three Immunization Injections at three-day Intervals.

Figure 1 shows the results of the influence of the A.P.P. on the precipitin value (titer) of egg white during the period of 57 to 59 days.

Three experimental groups of rabbits received three immunization injections containing both the A.P.P. and the antigen in their external ear veins at three-day intervals. In two of these three experimental groups, 24 hours after the second immunization injection (4 days after the first immunization injection) (in the following, if no mention is made of the number of days after injection, it should be considered as the number of days after the first injection), the precipitin value rose to 50 and 25 times of that of the

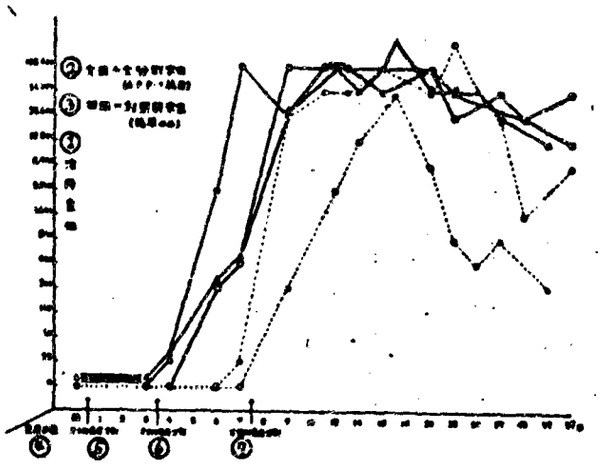

Fig. 1. Influence of the A.P.P. on the Precipitin Value of Egg White

[Legend]: 1) Precipitin Value; 2) Solid Line - Experimental Rabbits (A.P.P. + Antigen); 3) Broken Line - Control Rabbits (Antigen alone); 4) No. of Days Before Immunization; 5) First Immunization Injection; 6) Second Immunization Injection; 7) Third Immunization Injection.

control respectively. The precipitin value of the other experimental group rose to 200 times of that of the control group six days after immunization. After that, the precipitin value continued to rise, and it rose to 3200 to 200 times of that of the control immediately before the third injection or six days after the first injection. One of the groups showed a very rapid increase in the precipitin value, and it rose to 102,400 times of that of the control group. In the other two groups, the increase was slight on the day following the third injection; they increased to 102,400 to 25,600 times that of the check nine days after the first injection three days after the third injection). In all experimental groups, the increase continued for about two weeks after the first injection followed by a gradual decrease. Even after 57 to 59 days, a high precipitin value of 12,800 to 51,200 times of that of the control was observed.

In contrast to the above results, the two control groups which received only the antigen injections showed no precipitin formation even six days after immunization when all of the experimental groups had precipitin formations. The control groups showed 25 to 200 times of precipitin formation seven to nine days after the immunization. After this,

the control groups showed a rapid increase, almost to the same degree of that of the experimental groups for a short period of time. Some of the rabbits of the control group took a longer period of time in reaching the maximum, decreasing very rapidly, reaching 6,400 times 57 to 59 days after the first immunization. That is, the experimental groups started precipitin formation 25 hours to five days sooner than the control groups, and in the former the maximum precipitin value was reached sooner and maintained longer than in the latter.

Furthermore, in one group of rabbits which received only the A.P.P. injection, no increase in precipitin value has been observed.

2. Results with Three Injections at Three- to Six-day Intervals

An experiment was conducted using two experimental groups and one control group of rabbits which received the same immunization injections as in the previous experiment at three- to six-day intervals. Blood was collected from these groups three days after the first injection and continuing up to 26 days. Comparative measurements were made on the value of serum precipitin between the two groups, and the influence of the A.P.P. on the precipitin formation is shown in Figure 2.

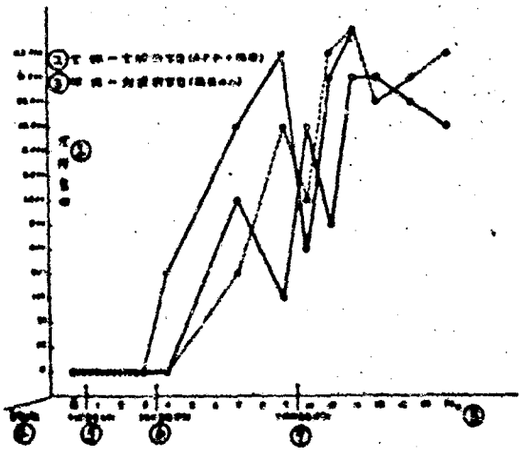

Fig. 2. Influence of A.P.P. on the Precipitin Value of Egg White

[Legend]: 1) Precipitin Value; 2) Solid Line-Experimental Rabbits (A.P.P. + Antigen); 3) Broken Line - Control Rabbits (Antigen Alone); 4) No of Days Before Immunization; 5) First Immunization; 6) Second Immunization; 7) Third Immunization; 8) After.

As in the previous experiment, in one of the two experimental groups, on the fourth day after the first injection (24 hours after the second injection) the precipitin value increased to 200 times of that of the check. It increased to 12,800 times after seven days, and to 102,400 times nine days after the first injection or right before the third injection. In the other experimental groups, the increase was delayed. It was 1,600 times seven days after the first injection, dropping rapidly to 100 times after nine days. The group showing the increase of 102,400 times nine days after the first injection showed an abrupt drop in the value to 400 times 24 hours after the third injection or 10 days after the first injection. After this, this group again showed an increase to 51,200 times on the third day after the third injection (12 days after the first injection), and to 204,800 times -- which was the maximum value -- on the fifth day after the third injection (14 days after the first injection). Then it decreased gradually, reaching 25,600 times after 19 days, and 12,800 times after 26 days. In another group, the value decreased to 100 times before the third injection, it increased to 12,800 times 24 hours after the third injection, and it again decreased to 800 times 12 days after the first injection. On the 14th day, however, it again increased to 51,200 times. The rabbits in this group began to have diarrhea from the 11th day after the first injection and died on the 16th day. This unfavorable condition may have caused the abnormal fluctuation in the precipitin value.

In the control group, no increase in precipitin value was observed four days after the first injection. On the seventh day, it rose to 200 times, and to 12,800 times on the ninth day. Ten days after the first injection, it decreased to 1,600 times, but it abruptly increased to 102,400 times on the 12th day. On the 14th day, it showed the maximum value of 204,800 times, which was followed by a decrease to 25,600 times on the 16th day.

In comparing the precipitin values between the experimental and control groups, one group which did not have the abnormal condition of the two experimental groups showed

increase in precipitin value three days earlier than the control group. In the former group, the increase was 12,800 times seven days after the first injection, at which time the control group had just started to show evidence of precipitin formation. Even in the abnormal group, the increase was 1,600 times seven days after the first injection, which was about eight times of that of the control group. On the ninth day, one (the group which died due to diarrhea) of the two experimental groups showed an abrupt decrease in the value, but both of these groups showed more conspicuous increases than the control groups. Therefore, it is thought that the A.P.P. seems to promote precipitin formation after the second immunization injection. It also appears that there was not too much difference between the two experimental groups after the third immunization injection, at which time the precipitin formation had already started.

3. Summary of the Above Experiments

According to the results of the above experiments, it appears that the A.P.P. clearly has the function of increasing the value of serum precipitin in rabbits.

B. Influence of the A.P.P. on the Quantity of Precipitin

In the previous experiment, the author proved the fact that the A.P.P. definitely influenced the increase of the value of serum precipitin. In this study, the quantity of serum precipitin influenced by egg white was measured and investigated, and the results are given in the following.

1. Three Immunizations at Three-day Intervals

Figure 3 shows the results of the study using three experimental groups of rabbits which received immunization injections of a mixture of antigen and the A.P.P. three times at three-day intervals, while the two control groups of rabbits received only the antigen injections.

In one of the three experimental groups, the precipitin formation started 24 hours after the second injection (four days after the first injection). In the other two groups, the formation started six days after the first injection, but there was not much change 24 hours after the third injection (seven days after the first injection). In the one group which showed precipitin formation earlier than the other two groups, the quantity increased to eight times four

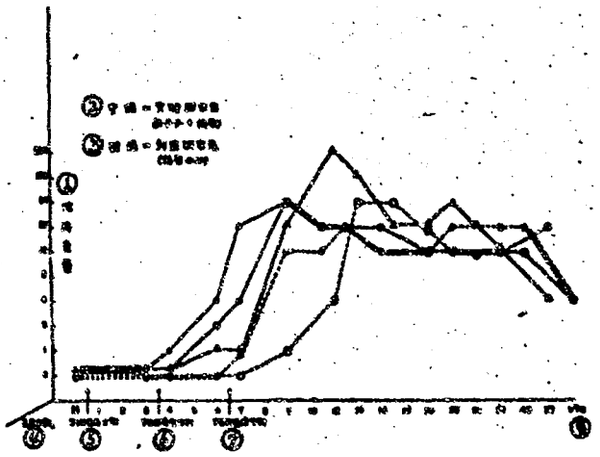

Fig. 3. Influence of A.P.P. on the Quantity of Precipitin of Egg White

[Legend]: 1) Precipitin Quantity; 2) Solid Line - Experimental Rabbits; 3) Broken Line - Control Rabbits; 4) No. of Days Before Immunization; 5) First Immunization; 6) Second Immunization; 7) Third Immunization; 8) After.

days after the first injection. Nine days after the first injection, all three groups showed a significant increase. At this time, the highest quantity of increase in the two groups was 64 times. In the other group, the increase was somewhat slower, and it reached 256 times 12 days after the first injection. In all of the groups, after the increase reached the maximum, it subsided gradually, but during the period of 20 to 23 days after the first injection it remained more or less constant, subsiding rapidly 37 to 43 days after the first injection.

In the control groups, the formation of precipitin started 24 hours after the third injection (seven days after the first injection) or nine days after the first injection. It increased gradually, reaching 32 and 64 times on the 13th and 14th day respectively. There was little change until 43 to 53 days after the first injection. These results show that in one of the three experimental groups, precipitin formation started three to five days earlier than in the control group, that in the other two groups the formation started one to three days sooner than the control group, and that in these two groups the increase reached 64 times nine days after the first injection. In one of the three experimental groups, the increase reached 256 times 12 days after the first injection. In contrast to this, precipitin formation started nine

days after the first injection in only one of the control groups. In the control groups, the formation of precipitin was one to six days behind that of the experimental groups, reaching 32 to 64 times 13 to 14 days after the first injection respectively. Beside the differences mentioned above between the two groups, there was no significant difference in the quantity of antibody formation between the two groups.

It should be mentioned here that there was no increase in the quantity of precipitin in the control rabbits which received only the A.P.P. injections.

2. Experimental Results with Three Immunization Injections at Three- to Six-day Intervals

In this study, measurements were made on the quantity of the precipitin from the two experimental groups and one control group of rabbits which receivedthree immunization injections at three- to six-day intervals. These data were obtained from the same experimental and control groups stated under A-2 above. The results of this study are shown in Figure 4.

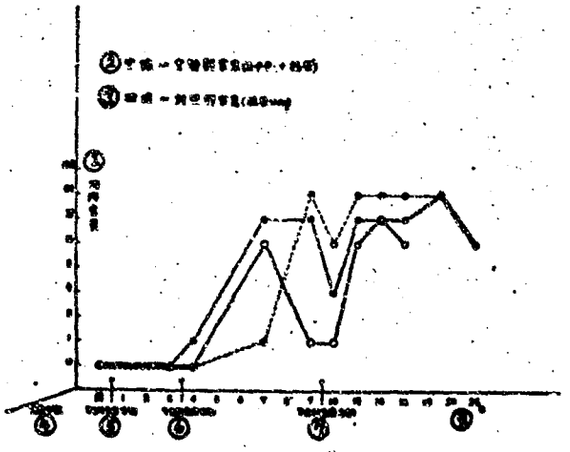

Fig. 4. Influence of A P. P. on the Precipitin Quantity of Egg White

[Legend]: 1) Precipitin Quantity; 2) Solid Line - Experimental Rabbits (A.P.P. + Antigen); 3) Broken Line - Control Rabbits (Antigen Alone); 4) No. of Days of Immunization (before); 5) First Immunization; 6) Second Immunization; 7) Third Immunization; 8) After.

In one of the two experimental groups, precipitin was discerned four days after the first immunization injection. It increased rapidly seven days after the injection, reaching 32 times which was maintained until nine days after the injection. In the other experimental group, the quantity of precipitin reached 16 times seven days after the injection, showing decrease on the ninth day. During the 24-hour period after the third injection, which occurred on the ninth day after the first injection and after the blood sample was taken, the quantity of precipitin increased to 32 times that of before the injection but it decreased to four times immediately after that. It again rose to 32 times 12 days after the first injection, maintaining this condition until the 16th day. In the other group, the quantity of precipitin remained low during the 24-hour period after the third injection. It rose to 16 times on the 12th day, and it reached 32 times on the 14th day, gradually decreasing to 16 times on the 16th day. As stated previously, this group had diarrhea from the 11th day after the experiment started and died on the 16th day.

In the control group, the appearance of precipitin started from the seventh day after the first injection. It reached 64 times on the ninth day but decreased after the third injection, followed by a gradual increase to 64 times on the 12th day, and continuing this condition until the 16th day after the first injection.

In the following, comparative results between the experimental and control groups are given. In one of the two experimental groups which did not show any abnormality, precipitin was discerned three days earlier than in the control group. This group had an increase of 32 times on the seventh day after the first immunization injection in comparison with the increase to double in the control during the same period of time. In the other experimental group, the increase reached 16 times on the seventh day after the first injection which is comparable to the 16-fold increase in the control group. On the ninth day after the first injection, one of the two experimental groups showed no change in the quantity of precipitin, while the other showed a decrease, which is contrary to the significant increase in the control group. From these results, it is thought that the A.P.P. promoted the production of precipitin in the rabbits which received immunization injections. Furthermore, it is thought that the A.P.P. does not seem to have a significant influence on the formation or retardation of the antibody after the start of precipitin formation in the animal body or after the third immunization injection.

3. Summary of the Above Results

From the results of the above experiments, it appears that the A.P.P significantly promotes the production of serum precipitin in rabbits.

C. A Comparative Study on the Promotion or Retardation of the Precipitin Value (Titer) and Quantity

In this experiment, the author studied the influence of the A.P.P. on the value and quantity of serum precipitin in rabbits. In the former, the old method of measuring the intensity of reactions was employed. For the study of the latter aspect (precipitin quantity) the most recent method of antibody measuring was employed as stated in the Introduction. Needless to say, the precipitation reaction is that which is a cartilagenous nature between a soluble antigen and a complementary antibody. Consequently, if one of the two complementary factors is supplied in a limited amount in the reaction, the reaction can not take place even though the other factor exists in a large quantity. In other words, in order to obtain a precipitation reaction, both the antigen and the antibody have to be of a certain minimum concentration. (In the following, this will be referred to as the minimum concentration of antigen and antibody.) Therefore, it is thought that the reaction of a minimum concentrated solution of an antigen will depend on the degree of concentration of the antibody solution. That is, if the degree of the antibody concentration is high, precipitation will occur even though the antigen concentration is low. In other words, under certain conditions, measurements can be made on the precipitin even though the antigen is diluted to a low concentration. In the following, the relationship between the value (titer) and the quantity of precipitin based on the results of the present experiment is discussed.

1. Results in the Case of Three Immunization Injections at Three-day Intervals

As shown in Figures 5 and 6, in general, the latency of both the value and the quantity of precipitin occurred during the same period. As shown in Figure 7, however, in some cases the increase in value preceded that of the quantity. The duration of time required to reach the maximum increase of the value and the quantity varied a great deal; in some cases the value preceded the quantity, in some cases both occurred at the same time, and in one case the former was behind the latter. In all cases, no parallel relationship

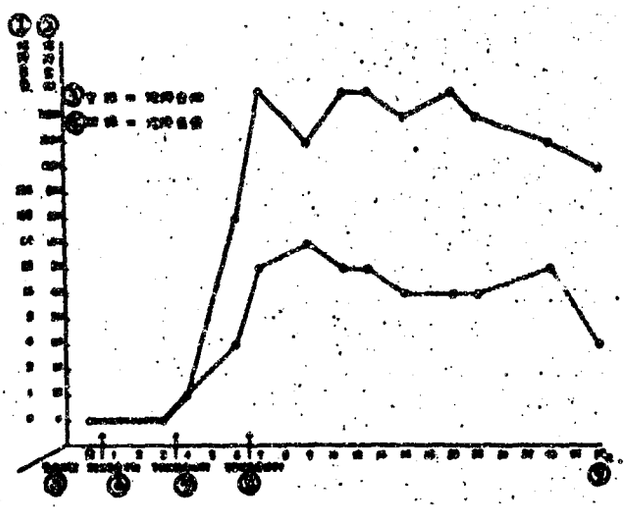

Fig. 5. Development in Value and Quantity of Precipitin in Immunized Rabbits (Example 1)

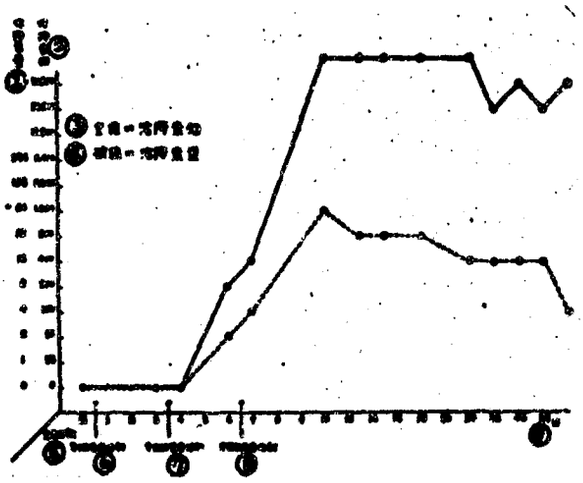

Fig. 6. Development in Value and Quantity of Precipitin in Immunized Rabbits (Example 2)

[Legend for Figure 5 and 6]: 1) Precipitin Quantity; 2) Precipitin Value; 3) Solid Line - Precipitin Value; 4) Broken Line - Precipitin Quantity; 5) Before No. of Days of Immunization; 6) First Immunization; 7) Second Immunization; 8) Third Immunication; 9) After.

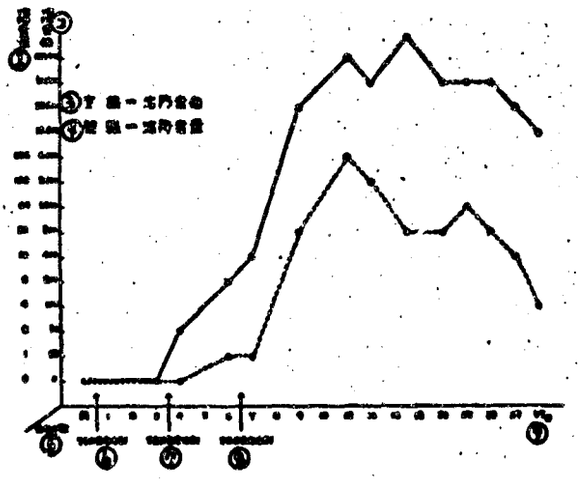

Fig. 7. Development in Value and Quantity of Precipitin in Immunized Rabbits (Example 3)

[Legend]: 1) Precipitin Quantity; 2) Precipitin Value; 3) Solid Line - Precipitin Value; 4) Broken Line - Precipitin Quantity; 5) Before No. of Days of Immunization; 6) First Immunization; 7) Second Immunization; 8) Third Immunization; 9) After.

has been found. In regard to the duration of continuation of the maximum increase after it reached this point, in most of the cases the precipitin quantity decreased gradually after this point. In contrast, the precipitin value kept its maximum for a longer period of time than the quantity. The mode with which the decrease took place in the value was slower than that of the quantity.

In Figures 8 and 9, the results of the two control groups are shown. These results are quite similar to those of the experimental groups. Here, again, there are no parallel relationships between the two factors, and as to the continuation period, there seems to be no definite relationship between the two.

2. Results in the Case of Three Immunization Injections at Three- to Six-day Intervals

As shown in Figures 10 and 11, the results given her are about the same as those given above. In both the experimental and control groups, there was a significant increase in the quantity of precipitin after the third injection.

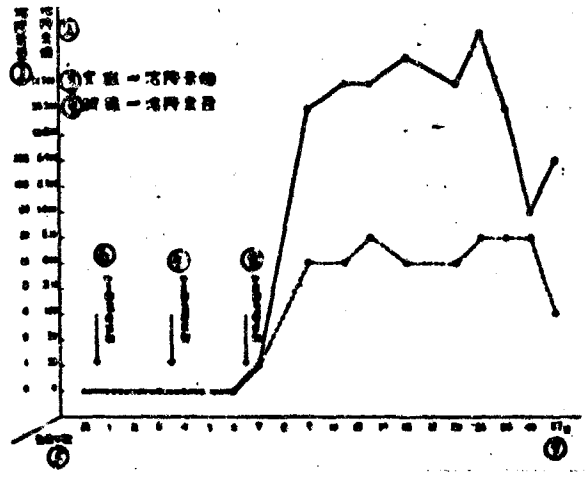

Fig. 8. Development in Value and Quantity of Precipitin in Immunized Control Rabbits (Example 4)

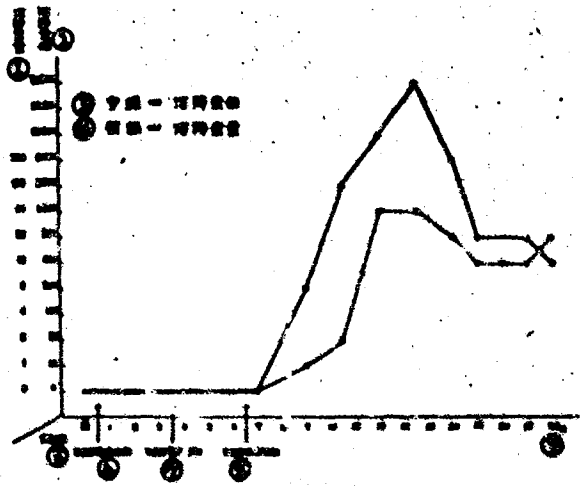

Fig. 9. Development in Value and Quantity of Precipitin in Immunized Control Rabbits (Example 5)

[Legend for Figures 8 and 9]: 1) Precipitin Quantity; 2) Precipitin Value; 3) Solid Line - Precipitin Value; 4) Broken Line - Precipitin Quantity; 5) Before No. of Days of Immunization; 6) First Immunization; 7) Second Immunization; 8) Third Immunization; 9) After.

It is interesting, however, to note that the precipitin value showed a significant increase in the control group in this period. (It should be mentioned, however, that the increase was slight during this period, having a high value previous to this.)

3. Summary of the Results Given in C

According to the results, the latent period of both the value and the quantity of precipitin is about the same, and both showed increase due to immunization injections, followed by a gradual normalization after a certain period of time. As reported by Matsubayashi and others, there is no inter-relationship between the two factors during the period of antibody production. At the same time, it is thought that the A.P.P. seems to have a greater influence on one of the two factors than on the other.

CHAPTER IV. SUMMARY AND DISCUSSION

The results of this experiment showed that when the A.P.P. and egg white antigen were introduced into rabbits at the same time, the former promoted the formation and increased both the value and the quantity of the egg white precipitin. In other words, the A.P.P. promoted the production of precipitin in rabbits.

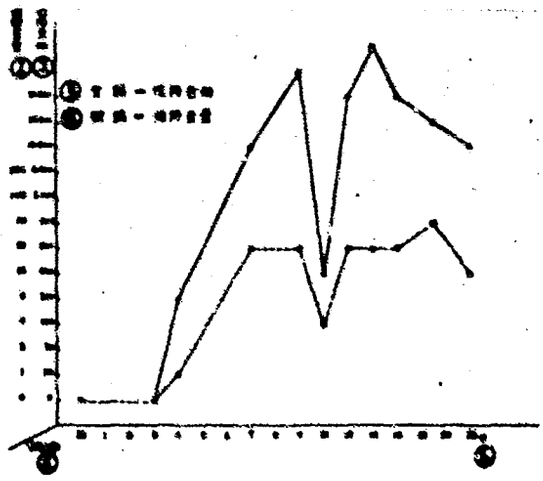

Fig. 10. Development in Value and Quantity of Precipitin in Immunized Control Rabbits (Example 6)

[Legend]: 1) Precipitin Quantity; 2) Precipitin Value; 3) Solid Line - Precipitin Value; 4) Broken Line - Precipitin Quantity; 5) Before No. of Days of Immunization; 6) After.

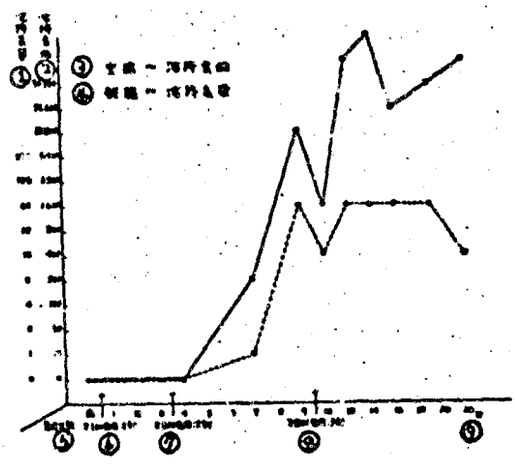

Fig. 11. Development in Value and Quantity of Precipitin in Immunized Control Rabbits

[Legend]: 1) Precipitin Quantity; 2) Precipitin Value; 3) Solid Line - Precipitin Value; 4) Broken Line - Precipitin Quantity; 5) Before No. of Days of Immunization; 6) First Immunization; 7) Second Immunization; 8) Third Immunization; 9) After.

The production of immunization substances in rabbits depends, to a great degree, on the individual characteristics of the rabbits. In this study, the author employed the same kind of rabbits of the same weight, and under similar experimental conditions, gave injections to the rabbits with antigens of definite quantities. Even so, it is impossible to discern the influences of these factors on the production of precipitin. The fact, however, that the use of the A.P.P. brought about the shortening of the latent period in all of the experimental groups can not be questioned.

Fukase[34] observed the function of promoting antibody production by the A.P.P. in his study on the coagulant of typhus organisms, on the hemolysin and coagulant of the red

corpuscle of mountain goat, and on the production of an antitoxin against diphtheria organism. this was true in the production of precipitin. The A.P.P. shortened the latent period from 24 hours to five days in comparison to the control, it increased the production of precipitin with a higher speed and vigor, and in some cases it prolonged the period of precipitin production.

The fact that injections of the A.P.P. alone did not increase the production of egg white precipitin in rabbits agrees with the results obtained by Fukase in his study on the production of coagulant of typhus organism and in other studies. These results also show that the A.P.P. exhibits its effectiveness only when it is used as a complementary substance along with an antigen.

Miyake[7], Hiraki, Naito[9], Takaishi[29,30], and others reported on the influence of the extracts of various organs on the production of serum precipitin when they are used as supplementary substances. These workers found that extracts of the same kind of white corpuscles, of bone marrow, and of immunized lymphatic gland promoted the production of serum precipitin. It is, however, not clear whether or not all these results were due to the A.P.P. The author recognized the presence of some substance which promotes the coagulant formation in typhus organism as does the A.P.P.

The quantity and the value of precipitin do not show a parallel relationship, and Ogata and others reported that the former shows more normalcy than the latter. In this experiment, both the experimental and control groups revealed similar results. In one of the experiments in which the A.P.P. was used, the appearance of precipitin value preceded that of the quantity. Besides this example, the A.P.P. did not seem to have any different effect on either one of the two.

The fact that lymphatic gland[44,45,46,47,48,49] is the most important organ in producing antibody has been advocated by many workers. As to the mechanism of this production, there are many theories. As to the mechanism of this production, there are many theories. The lymph corpuscle theory has been advocated by Ehrlich and Harris[51], Harris[52], Dougherty, Chase and White[53], Harris, Mertens and Ehrlich[54]. The cell plasma theory has been advocated by Amano[55,56], by Fargraeus[58], by Ehrlich, Drabkin and Forman[57], and by many others. Furthermore, there is the reticulo-endothelium system theory which has been advocated by Sabin[69], by Professor Kimura[49] and by many other students. At present, there is no single theory on which all workers agree.

According to Shiraki[59,60], during the time of the production of precipitin after immunization with the A.P.P., there occurs a great change in the lymph corpuscles. Especially, at the time of increase in the antibody, the number of lymph corpuscles of the blood decreases in comparison with the time previous to this. During this time, many lymph corpuscles were broken down; they appeared to be weaker, and many of their shapes became theorny. Hinoshita[61] studied the conditions of lymph corpuscles under similar conditions by staining with neutral red and janus green. He observed many young lymph corpuscles and an increased activity in the lymph system under these conditions in comparison with the activities during a normal time. It has also been reported that in some instances the lymph plasma increased during the period of increased antibody and that there was no significant change in the reticulo-endothelium system. Both of these workers pointed out that there is a close relationship between the increase in antibody and lymph corpuscle.

There are many theories on the mechanism in the formation of antibody -- how it is synthesized and how it is different from the serum protein. Regarding this subject, there is the side chain theory of Ehrlich. Later, Haurowitz[62] corrected this theory, and claimed that the antigen injected into a living body is immediately carried to the organs of the reticulo-endothelium system where it is accumulated. This accumulated antigen forms its own foundation there, interfering with the formation of regular serum globulin with amino acid, and forming a new kind globulin -- the new antigen -- which has the ability to react even through an empty space and against electric charges. Alexander[67] thinks that antigen inside the cell creates a special catalysis which contributes to the formation of antibody which is sort of a combination of catalyzer. Upon combination of this catalyzer-antibody and antigen, a new antibody is formed. Pauling[63], on the other hand, considers that antigen is formed by a special chain arrangement of a long peptide which is formed through regular synthesis of globulins in the presence of an antigen. Besides, Jordan[66] advocated the theory of self-catalysis among antibodies themselves. Oka[64,65], however, advocates a theory which is different from all others. According to his theory, antibody is formed through the influence of antigen during the regular process of serum globulin formation with amino acid. He further thinks that the synthesis of protein is accomplished by an emzyme which is present in the cells in which serum globuli are formed. Fukase seems to have the opinion that the enzymes which are present in the antibody-producing organs change the direction of regular protein formation at the time of antigen invasion and instead

form antibody. He based this theory on the finding of the A.P.P. in lymph glands.

The fact that the A.P.P. functions on the destruction and renewal of lymph corpuscles has been proven through the studies made by Shiraki and Hinoshita. The matter as to what relationship there is between the A.P.P. and the antibody production mechanism can be better understood through the experiments of White[68,70] in which he found that the hormone of spleen caused antibody formation in broken lymph corpuscle.

CHAPTER V. CONCLUSION

The following is the conclusion on the influence of the A.P.P., a name given by Fukase on the albumin precipitin production in rabbit.

1) When A.P.P. is administered to rabbit with albumin antigen, it increases both the value (titer) and the quantity of precipitin, and it also shortens the latency period of precipitin formation.

2) There is no direct parallel relationship between the value or titer and the quantity of precipitin in the same immunized rabbit.

In concluding, the author wishes to express his deepest gratitude to Professor Kukuchi (new emeritis professor) and to lecturer Fukase (now assistant professor) for their guidance and for editing this paper.

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